CERTIFICATE OF MAILING

I hereby certify that this paper is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria VA 22313-1450, on May 5, 2006.

Signature Theodore J. Leitereg **Date** 5/5/06

Name: Theodore J. Leitereg

PATENTS
Attorney Docket No. 7459

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Yi Feng Zheng, *et al.*

Serial No.: 10/736,004 Group Art Unit: 1641

Filed: December 15, 2003 Examiner: Shafiqul Haq

Title: Assays for Entactogens

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

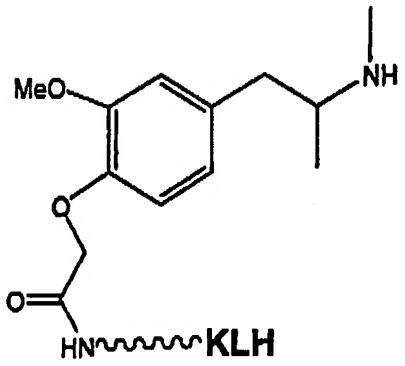
DECLARATION OF INVENTOR PURSUANT TO RULE 37 CFR 1.131

I, Yi Feng Zheng, declare that the following statements are true of my own knowledge, or are believed to be true if stated to be based upon information and belief, and if called upon, I would testify to the truth of these statements.

I am a chemist with Dade Behring Inc., the assignee of the above-referenced U.S. Patent Application, Serial No. 10/736,004, filed December 15, 2003 (hereinafter "Application").

I am one of the co-inventors (hereinafter 'Applicant') named in the Application. I have read the Application and I am familiar with the contents of the Application. Moreover, I have read an Office Action from the U.S. Patent and Trademark Office (USPTO) mailed February 6, 2006 (hereinafter "Office Action"), and I am familiar with the contents of the Office Action.

Prior to January 23, 2002, my co-inventors and I prepared the following compound:



Attached hereto as Appendix A are notebook pages 26-33 from my Laboratory notebook #4908 at Dade Behring Inc. The notebook pages show the details of the preparation of the above compound.

All of the dates blacked out in Appendix A were prior to January 23, 2002.

I further declare that all statements made herein are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent application Serial No. 10/736,004, or any patent issued thereon.

Dated on this 4 th day of May 2006 at Wilmington DE.

Yi Feng Zheng

Yi Feng Zheng

APPENDIX A

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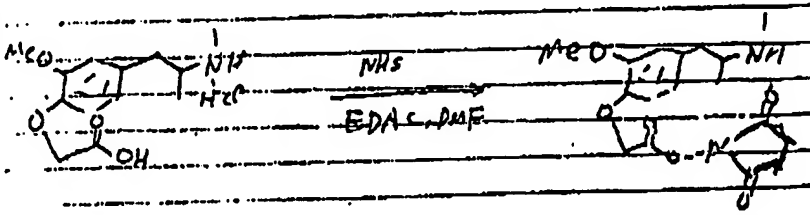
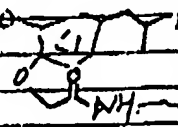


↓ KLH – NH_2

↓ PBS buffer pH=8.0



(4908-26)

Reagents	MW	Weight	mmols	Sources
S-4908-12	289.74	10 mg	0.0345	page 12
EDAC·HCl	191.71	20 mg	0.1043	
NHS ester	115.09	19 mg	0.165	
DMF	(0.6 mL)			
KLH	5.5×10^6	20 mg		
PBS	buffer (pH=8.0, 0.1 M, 6 mL)			

Procedure: To a solution of S-4908-12 (10 mg) in DMF (0.6 mL) was added EDAC·HCl (20 mg) and NHS (19 mg). The reaction mixture was stirred under argon at room temperature for 2.5 hours. The activated ester was added dropwise via a syringe to a well prepared solution of KLH (20 mg) in NaH_2PO_4–Na_2HPO_4 buffer (6 mL, pH=8.0, 0.1 M). The pH value was changed during the addition. 0.1 N NaOH was used to adjust pH value to 8.0. After the complete addition, the reaction was allowed to stir overnight at 4°C (16 hours). The reaction mixture then was ready to dialyze. The 3 L dialyzing buffer solution was prepared from

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[redacted] Dulbecco's phosphate buffer saline (Sigma, Cat No D-1408, Lot No: 48H2339) in a ratio of 400 mL of buffer mixing up with 2600 mL of DE Water.

10 mM Na_2HPO_4 – NaH_2PO_4 buffer (pH = 7.0) was prepared from stock solution of 0.2 M NaH_2PO_4 and Na_2HPO_4.

The KLH-Immunogen (reaction mixture) was dialyzed again Dulbecco's phosphate buffere saline.

3 L, 4°C overnight (~16 hours).
3 L 4°C 24 hours.
3 L 4°C 40 hours

10 mM NaH_2PO_4 – Na_2HPO_4 buffer Solution (pH = 7.0)

3 L 4°C 3 hours.
3 L 4°C 4 hours.

14 mL of Immunogen was obtained denoting as 4968-27

Determination of the concentration of KLH-Immunogen by using BCA Protein Concentration Assay

[redacted] 1) Working Reagent

Reagent A for BCA protein Assay (Pierce Cat No: 23224 Lot No: 9510206)
Reagent B " " " " (Pierce Cat No: 23224 Lot No: 9508-2567)

Reagent A (25 mL) + Reagent B (0.5 mL) = 25.5 mL working Reagent (green)

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2) Sample Preparation in NaH_2PO_4 - Na_2HPO_4 buffer (pH=7.0) and Sodium borate solution (0.1M)

0.1 ml of Immunogen in 0.2 ml of 0.1 M Sodium borate $\frac{1}{3}$X

3.) KLH standard

#1 1.30 mg/ml in 0.1 M Sodium borate
#2 0.65 mg/ml in 0.1 M Sodium borate } duplicated samples.
#3 0.325 mg/ml in 0.1 M Sodium borate

4) Samples + Standards + Blank = 2+4+1 = 9 tubes.

each tube contains 0.1 ml of immunogen or standard or blank dilute with 2ml of working Reagent. Incubate at 40°C for 30 minutes.

5) UV-vis checked OD at 562 nm

	OD λ=562 nm	Concentration
#1	1.002	1.30 mg/ml
#2	0.5378	0.65 mg/ml
#3	0.26675	0.325 mg/ml
Immunogen	0.564	0.12 mg/ml

→ see Standard curve in page: 29

$y = 0.0326 + 0.74756X$ $R = 0.9995$

$0.561 = 0.0326 + 0.74756X$

$X = 0.7068$

Immunogen concentration = $3 \times 0.7068 = 2.12$ mg/ml.

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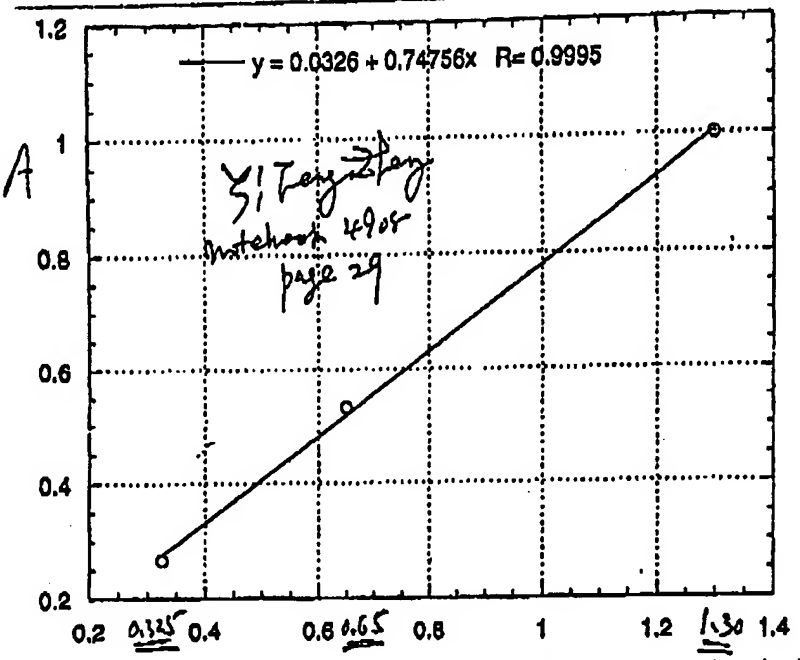
OD = A at λ = 562 nm



Determination of Hapten Number for KLH Immunogen

Reagents
1) 0.1 M Sodium borate
2) 0.1% (w/w) TNBS (2,4,6-trinitrobenzene sulfonic acid)
3) 1 M HCl
4) 10% (w/w) SDS (Sodium dodecanoate) Solution

1) 0.1 M Sodium borate (100 mL) pH = 9.32 $Na_2B_4O_7 \cdot 10H_2O$ MW = 381.37

$0.1\ M \times 0.1\ L = \frac{X}{381.37}$ X = 3.81 g (100 mL H_2O)

2) 10% SDS 10 mL, $CH_3(CH_2)_{11}OSO_3Na$ MW = 288.22
1 g of SDS in 9 g (9 mL) of H_2O)

3) 0.1% TNBS Solution · TNBS = MW = 293.2
TNBS·3H2O MW = 383.2

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20 mg × 388.2 / 295.2 = 26.1 mg (20 mL H_2O)

4) Sample of KLH Immunogen at a concentration 2.12 mg/mL
- 0.5 mL of KLH Immunogen #908-26
- 0.5 mL of 0.1 M Sodium borate
- 0.5 mL of 0.1% TNBS
- 40°C Incubation for 2 hours
- 0.5 mL of 1M HCl
- 0.5 mL 10% SDS.
- UV-vis range 200-480 nm, checked OD at λ=340nm

5) KLH
- 0.5 mL of KLH solution of 1.3 mg/mL
- 0.5 mL of 0.1 M Sodium borate
- 0.5 mL of 0.1% TNBS
- 40°C incubation for 2 hours
- 0.5 mL of 1 M HCl
- 0.5 mL of 10% SDS
- UV-vis range 200-480nm, checked OD at λ=340nm

6) Blank Solution
- 1.0 mL of 0.1 M Sodium borate
- 0.5 mL of 0.1% TNBS
- 40°C incubation for 2 hours
- 0.5 mL of 1M HCl
- 0.5 mL of 10% SDS
- UV-vis range 200-480 nm

7) UV-vis scanned at 200-480nm, checked at λ=340nm

Sample	OD λ=340 nm	Concentration
KLH	0.9674	1.30 mg/mL
KLH Immunogen	0.3580	

see next page

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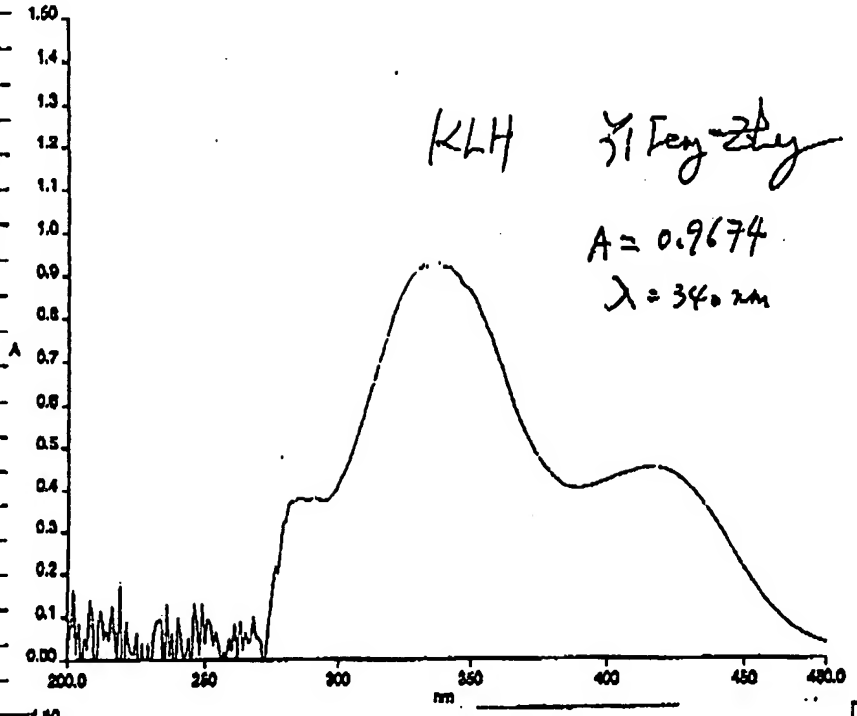
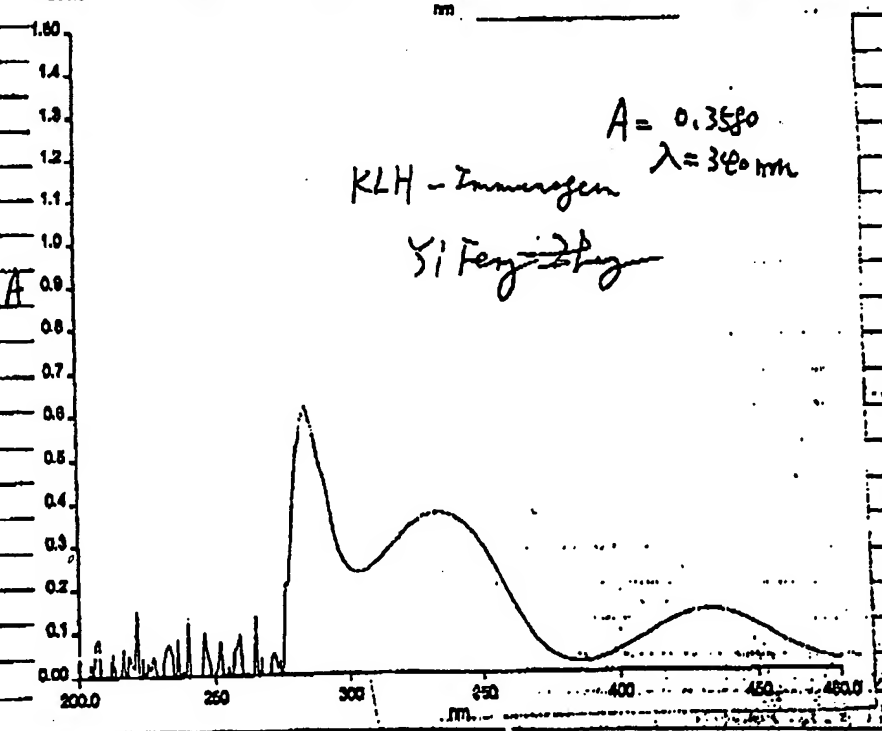
[redacted]



KLH Yi Feng Zhang

A = 0.9674

λ = 340 nm



A = 0.3580

λ = 340 nm

KLH - Immunogen

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[redacted]

Hapten Number Calculations

$$[KLH] = \frac{1.30\ mg/mL}{5.5 \times 10^6} \times 0.2 = 4.73 \times 10^{-8}$$

$A = \varepsilon b c$ $\varepsilon_{340} = 1.1 \times 10^4$ (TNBS) $b = 1$ cm

$$[C]_{KLH\text{-}TNBS} = \frac{0.9674}{1.1 \times 10^{-4} \times 1\ cm} = 8.79 \times 10^{-5}$$

TNBS reacted with KLH

$$\frac{8.79 \times 10^{-5}}{4.73 \times 10^{-8}} = 1858$$

Immunogen: KLH Immunogen $= \frac{2.12\ mg/mL}{5.5 \times 10^6} \times 0.2$

$= 7.71 \times 10^{-8}$

$A = \varepsilon b c$

$$[C]_{KLH\ Immunogen} = \frac{0.358}{1.1 \times 10^{-4}} = 3.25 \times 10^{-5}$$

TNBS reacted with NH_2 on Immunogen

$$\frac{3.25 \times 10^{-5}}{7.71 \times 10^{-8}} = 421.5 \approx 422$$

Hapten No $= 1858 - 422 = 1436$

Hapten% $= \frac{1436}{1858} \times 100\% = 77.3\%$

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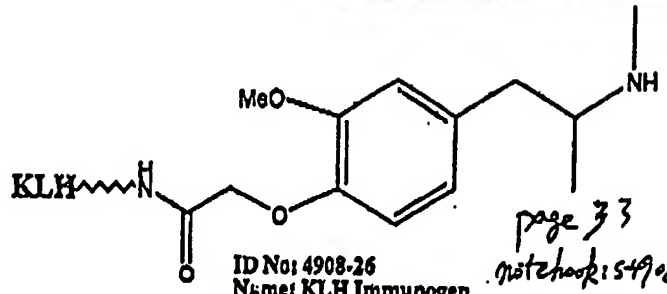

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notebook: 5408

ID No: 4908-26
Name: KLH Immunogen
Conc: 2.12 mg/ml
Buffer: 10 mM Na_2HPO_4-NaH_2PO_4, pH ≈7.0
Hapten No: 1436
Hapten%: 77%
Amount: 13 mL

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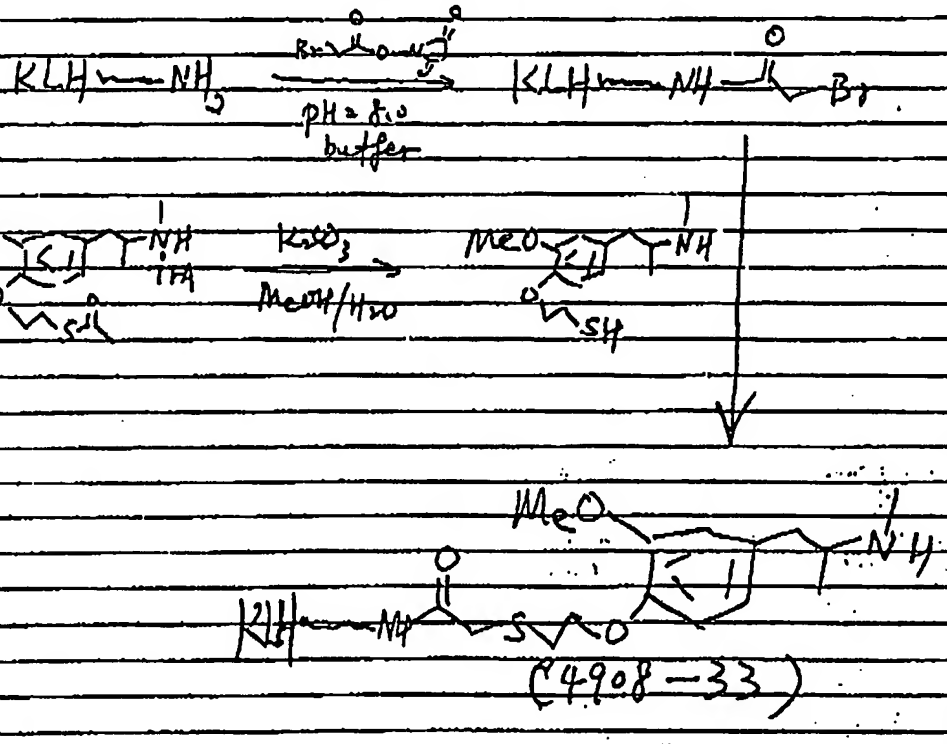

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